Letter to the Editor

Effects of In Vivo T-Cell Depletion on Immunity to *Eimeria falciformis*

I read with great interest the report of Stiff and Vasilakos on the effects of in vivo T-cell depletion on immunity to *Eimeria falciformis* (8). Their conclusion that depleting effector T-cell function results in an abrogation of protective immunity to *E. falciformis* is important but is contingent on their assertion that T cells are functionally deleted in the anti-Thy-1.2 (and complement)-treated mice. This the paper does not demonstrate. In fact, the data indicate only a mild reduction (~50%) in mitogen-driven T-cell responses following intravenous injections of anti-Thy-1.2 ascites and rabbit complement. These proliferative responses, ranging from 5.5×10^4 to 7.5×10^4 cpm in the data presented, are indicative of very strong T-cell function in "T-cell-depleted" mice. I suggest that analysis of Thy-1-expressing cells in these mice by flow cytometry would yield values not markedly lower than those observed in controls.

Secondly, the mitogen response appears to have been measured only at 24 h posttreatment. Most investigators find that chronic repetitive administration of monoclonal antibody is necessary to maintain satisfactory depletion of lymphocyte subsets in vivo. Characterizing T-cell function at 24 h and following disease progression over the following 20- to 25-day period is very much open to question. A correlation between loss of T-cell function in vivo and abrogation of protective immunity requires the demonstration that T-cell immunity is not present (or is very greatly reduced) throughout the time course of the study.

Selective depletion methods using antibodies directed against subset-specific determinants (administered in the absence of xenogenic complement) is a technique of great utility in delineating the in vivo role of different lymphocyte subsets. For reasons unknown, some lymphocyte populations are relative easily depleted (i.e., CD4 T cells with GK 1.5 [2] or YTS 191.1 [1], CD8 T cells with 2.43 (7) or YTS 169.4 (1), and B cells with polyclonal anti-μ [3, 4, 6]), while others are comparatively resistant (i.e., CD3 T cells with 145-2C11 alone [5] or T cells with anti-Thy-1). The mechanism by which functional deletion of these lymphocyte subsets is achieved remains unresolved but appears to be almost certainly complement independent.

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Author's Reply

As was stated in our discussion, "This in vivo Thy-1.2 cell depletion resulted in approximately a 50% reduction of a ConA-induced spleen cell lymphoproliferation. Since this lymphoproliferation was only 50%, we assume that Thy-1.2 depletion was not complete." However, this amount of T-cell depletion by monoclonal antibody (MAb) treatment did deplete natural acquired immunity to a level that not only abrogated immunity to coccidia but also prolonged the life cycle of the parasite in the host, as demonstrated by increased numbers of oocysts over longer periods of time. Instead of cell numbers (flow cytometry), we decided that measurement of T-cell function by concanavalin A mitogenesis was a more important functional parameter to study. The 24-h treatment with the MAb may not have been enough time to cause a physical depletion of the numbers of T cells, but their function was obviously impaired, as witnessed by the break in solid immunity to the coccidia (i.e., in none of the immune mice did parasites replicate). A chronic exposure to the MAb may be necessary to abolish the T cells, but functional interference may occur (for sure in this case) soon after administration of the antibody.

Also, since this treatment abrogated immunity, why wouldn't you measure the disease progression? Our data demonstrated a reduction in T-cell function by the MAb caused conditions in the host for better and prolonged replication of the parasite (thereby affecting disease pathogenesis). Even though we did not demonstrate a sustained depletion of T cells throughout the 20 days by the lymphoproliferation assay (not 25 days since the mice were given MAb 4 days postchallenge as well [see Materials and Methods]), doesn't the study of disease extension and severity tell one about the nature of the effect of this T-cell depletion on disease pathogenesis as well?

Regarding the timing of the concanavalin A experiments 24 h following treatment, this corresponded to the time when the parasites were given to similarly treated mice that were immune to coccidia. This was done to correlate the amount of depletion that occurred 24 h following administration of the MAb with the time the mice received the coccidia.

Finally, if this mechanism of depletion with MAb is unresolved, our studies included complement for both the

controls and treated mice and show that the complement alone did not deplete mice of their immune functional status in relation to the coccidia. These studies did not include experiments identifying the mechanism of depletion (i.e., with and without complement).

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